Clinical rheumatology, 1991, **10**, N° 2 151-154

Effect of Interleukin-1 on Hyaluronate Synthesis by Synovial Fibroblastic Cells

Y.T. KONTTINEN, H. SAARI, D.C. NORDSTRÖM

Summary **Interleukin-1 (IL-1) stimulates fibroblast-mediated hyaluronate (HA) synthesis in vitro. In the present study the degree of polymerization of such HA was studied using HPLC (high performance liquid chromatography) with a size exclusion column combined with ^{125}I-HABP assay used to measure the HA concentration in various HA molecular weight fractions separated using HPLC. IL-1 stimulated HA was more polydisperse than that produced by resting fibroblasts with a molecular weight varying from more than 4×10^6 daltons to less than 7.1×10^3 daltons. This IL-1 effect may contribute to the low molecular weight HA produced by freshly explanted arthritic synovial tissue and to the low viscosity of arthritic synovial fluid in vivo.**

Key words: **Interleukin-1, Fibroblast, Hyaluronate.**

INTRODUCTION

Hyaluronate (HA) is a high molecular weight non-sulfated polymer of N-acetyl-glucosamine and glucuronic acid disaccharide repeat unit (1). Synovial fluid is rich in HA synthesized by synovial fibroblasts and lining cells (2,3). In inflammatory arthritides, e.g. rheumatoid arthritis (RA), viscosity depends on HA concentration (4,5) so that the relative viscosity is exponentially proportional to HA concentration in individual samples, the rise in viscosity being particularly steep when synovial fluid HA concentration exceeds 2-2.5 mg/ml (6). Quantitative analysis shows that the scatter in different synovial fluid samples (with varying degrees of HA polymerization) around this HA concentration-synovial fluid viscosity curve is explained by degree of HA polymerization (5): depolymerization of HA contributes to the decreased synovial viscosity in inflammatory arthritides (4-6). It has also been described that freshly explanted synovial tissue produces in arthritis low molecular weight HA in vitro (7). Furthermore, immunoperoxidase-autoradiography double-labeling studies (8) and immunohistochemical evidence for procollagen containing fibroblasts in such tissue (9) suggest local fibroblast activation in RA in situ in inflammatory synovial tissue.

The reason for depolymerization of synovial fluid HA is unknown. It is possible that high molecular HA is degraded in arthritis, in particular by oxygen-derived free radicals (10,11,12) produced in situ by ischemia/reperfusion injury (13) and by NADPH oxidase associated with inflammatory phagocytes (14). In addition to this local degradation, it is possible that activated fibroblasts produce low molecular weight HA (7). Interleukin-1 (IL-1) is an important inflammatory mediator able to activate fibroblasts to increased HA synthesis (15-20). There are no direct studies, however, on the effect of IL-1 on the molecular weight of fibroblast synthesized HA. We therefore decided to study the molecular weight changes induced by IL-1 in vitro in synovial monolayer cultures.

Division of Rheumatology, Fourth Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland.

MATERIALS AND METHODS

Fibroblast explantation.

Knee joint synovial tissue samoles from RA (21) (four letter codes) patients (n=7) and osteoarthritis (22) (OA; three letter codes) patients (n=2) were used to establish fibroblast lines by explantation as described in detail elsewhere (8). Briefly, the tissue samples were cut into 3-5 mm^3 pieces and explanted onto 25 cm^2 tissue culture flasks (Nunclon Delta, Roskilde, Denmark) in Dulbecco's modified Eagle minimal essential medium supplemented with 1% (v/v) heat-inactivated fetal calf serum (FCS, Flow Laboratories, McLean, VI) and antibiotics (100 IU/ml penicillin-G and 50 µg/ml streptomycin sulfate), and were then cultured in humidified 5% CO_2 in air. The cell lines used in the experiments were passaged 6-11 times by detaching the fibroblasts

with 0.5% trypsin in Hank's balanced salt solution without Ca^{2+} and Mg^{2+}. The cultures were free of mycoplasma contamination as screened with Hoechst 33258 flurochrome (23).

Fibroblast stimulation.

For the experiments the detached fibroblasts were planted on tissue culture slides (growth area 1.8 cm^2 per chamber; Lab-Tek 4804, Miles Scientific, Division of Miles Laboratories, Naperville, IL) to a final volume of 1.0 ml. Fibroblast clture medium was or was not supplemented for 24 hours with purified IL-1 (Genzyme/Koch-Light, Suffolk, UK) to a final concentration of 1 IU/ml unless otherwise indicated.

^{125}I-HABP assay.

^{125}I-HABP assay (HABP = hyaluronate binding protein (HA 50 test®, Pharmacia Diagnostics AB, Uppsala, Sweden) is a sensitive radiometric assay for the detection of hyaluronate from serum and from body fluids and tissue homogenates (24) and was used in the present study as described in detail elsewhere (25).

High performance liquid chromatography.

Exclusion chromatography was performed in a Waters Associated chromatography pump (Model 6000A with a sample loop of 50 µl) equipped with a Toya Soda TSK 6000 PW column (60 cm × 7.5 cm) with a guard column of the same material (6,26). Peak elutions were detected using a LKB 2151 variable wavelength monitor (wavelength 206 nm) with a LKB 2220 recording integrator. The elution buffer was 50 mM sodium phosphate, pH 6.5, and the flow rate was 1.0 ml/min. All measurements were done at room temperature. Calibration of the column was done by using approriate molecular weight standards (Healon®, Pharmacia Diagnostica AB, Uppsala, Sweden) which were studied by the manufacturer by using the low angle laser light scattering method. The specificity of the method has been established earlier by using HA standards, by showing sensitivity to hyaluronidase and by measurement of HA in various elution fractions by ^{125}I-HABP assay mentioned above.

RESULTS

The presence of IL-1 increased, as expected, the concentration of HA produced into the fibroblast monolayer supernatant (Fig. 1). To study the molecular weight of the HA synthesized in the absence or presence of IL-1, HPLC was used to fractionate the fibroblast monolayer supernatant sample HA in the various fractions measured using the sensitive ^{125}I-HABP test. It was found that the HA synthesized in the presence of IL-1 was more polydisperse than the HA synthesized in the absence of IL-1 (Fig. 2): both at the high molecular weight (low retention time) and low molecular weight (high retention time) HPLC fractions the HA concentration in the nontreated synovial fibroblast cultures reaches baseline before the IL-1 stimulated cultures. This difference covers one or two fractions at most at

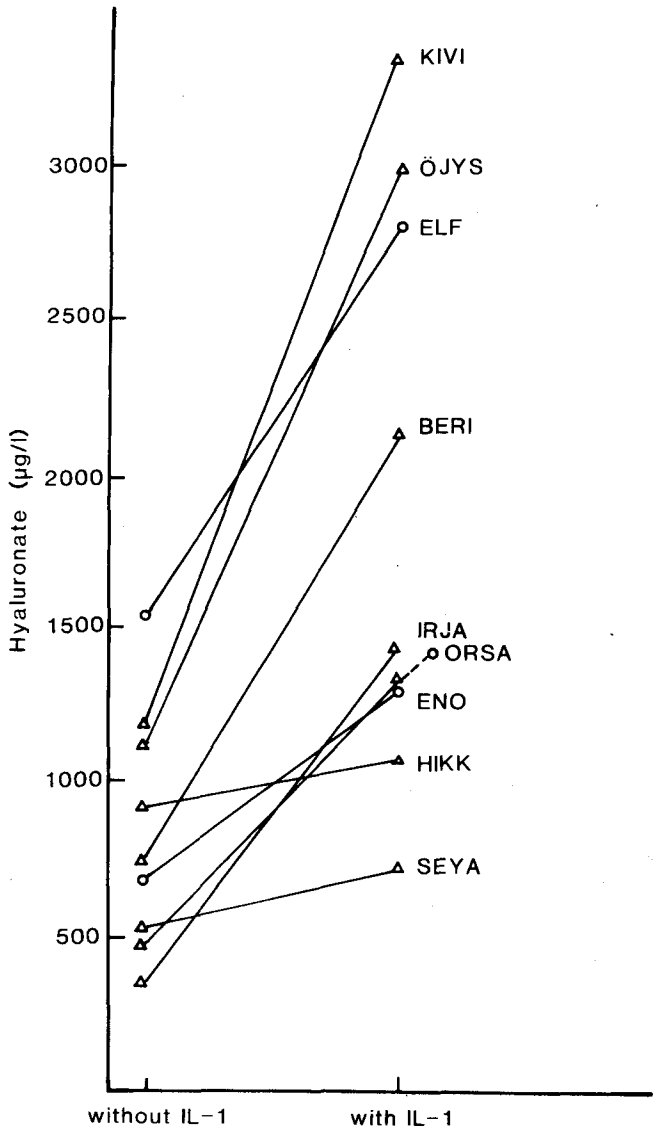

Fig. 1: Hyaluronate concentration in the fibroblast monolayer culture supernatant in the absence or presence of interleukin-1 (1 IU/ml). The difference between interleukin-1 treated and nontreated cultures was statistically significant ($p<0.01$, Mann-Whitney test). Four-letter combinations indicate fibroblast lines derived from rheumatoid synovial tissue; three-letter combinations indicate lines derived from osteoarthritis synovial tissue.

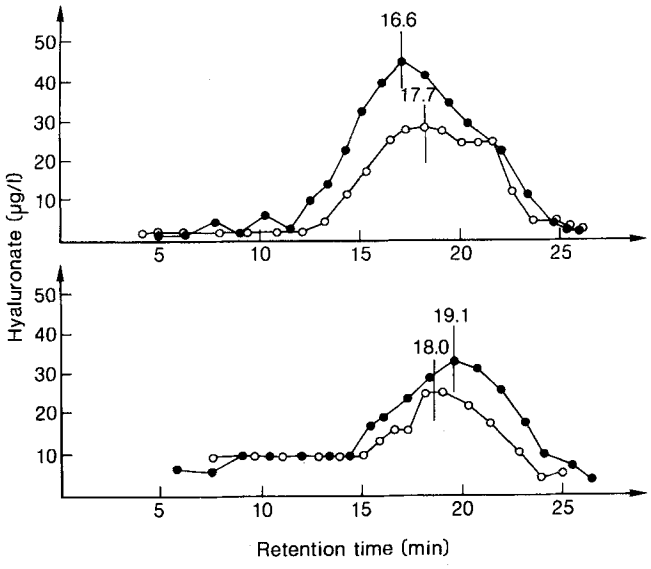

Fig. 2: Elution profiles of hyaluronate (HA) produced in the presence (•) or absence (○) of interleukin-1. Two experiments, both performed using RA derived synovial fibroblast lines (upper panel, lower panel). High performance liquid chromatography (HPLC) with size exclusion column was used for fractionation and HA in various fractions was measured using ^{125}I-HABP assay. Retention time in minutes in HPLC is given on the X-axis and HA concentration as measured in each HPLC fraction by ^{125}I-HABP assay is given on the Y-axis. The vertical bars refer to the retention time of the peak fraction with the highest HA concentration.
Toya Soda TSK 6000 PW column (60 cm × 7.5 cm) used in HPLC is a size exclusion column meaning that HA is eluted based on its hydrodynamic volume i.e. high molecular weight HA is eluted with low retention time, whereas low molecular weight HA is retained for longer. Therefore, the figure also depicts the molecular size of HA affected by interleukin-1: polydispersity is increased in low as well as in high molecular weight fraction. Note that the relationship between retention time and molecular weight is logarithmic so that small differences in the retention time reflect large changes in the molecular weight. According to molecular weight calibration of the HPLC column used, the molecular weight of HA produced varied within wide limits from more than 4 × 10^6 daltons to less than 7.1 × 10^3 daltons.

each end of the molecular weight spectrum and is also clear considering the loglinear relationship between molecular weight and retention time, which means that compounds with great molecular weight differences elute in a very narrow retention time range. The molecular weight of HA synthesized varied within wide limits from more than 4 × 10^6 daltons to less than 7.1 × 10^3 daltons.

DISCUSSION

It has been shown earlier (15-20) and is here confirmed by ^{125}I-HABP assay that IL-1 increases fibroblast-mediated HA synthesis. ^{3}H-glucosamine has been used to trace the molecular weight profile of HA synthesized by IL-1 stimulated fibroblasts in vitro (27). In this technique, ^{3}H-glucosamine is incorporated into the HA molecule synthesized and secreted into the culture medium. After separation of HA from sulfated glycosaminoglycans (28) elution profiles were studied by conventional gel chromatography and measurement of the radioactivity in various fractions. This procedure includes a lot of sample manipulation and this again might lead to artefactual changes and partial degradation of HA during the experimentation. The difference may also be related to the use of radiolabel material: differences in specific activity of HA synthesized in the presence and absence of IL-1 would lead to differences in the elution profiles depending on whether radioactivity (27) or the total HA molecule (present study) is being monitored. We therefore used a different approach by stimulating cultured fibroblasts in vitro with IL-1 and measuring the native, non-radioactively labeled HA in the supernatant using HPLC with size exclusion column. It was possible to study the elution profile and molecular weight distribution of HA produced by resting and IL-1 stimulated fibroblasts in monolayer cultures. It was found that HA synthesized in the presence of IL-1 was more polydisperse than the HA synthesized in the absence of IL-1. Furthermore, according to our findings the molecular weight of HA synthesized by IL-1 stimulated fibroblasts varied within wide limits from more than 4 × 10^6 daltons to less than 7.1 × 10^3 daltons. Therefore, the IL-1 effect may contribute to the earlier observation that rheumatoid synovial fibroblasts produced increased amounts of HA with decreased molecular weight in vitro (29,30). Our observation is also in line with that of Dahl and Husby who noticed that in vitro explanted rheumatoid synovial tissue pieces release low molecular weight HA (7).

Aknowledgement: This study was supported by the Sigrid Juselius Foundation, Arthritis Foundation in Finland, the Finnish Academy and the Hospital for Joint Diseases, Orthopedic Institute, New York, NY.

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Received: 27 August 1990; Revision-accepted: 12 November 1990
Correspondence to: Dr. Yrjö T. KONTTINEN,
Institute of Molecular Immunology, c/o Prof Robert J. Winchester, Hospital for Joint Diseases, NYU Medical School, 301 East 17th Street, New York, NY 10003, USA.